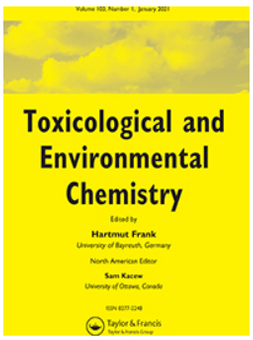

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Zahra Abpeikar, Roohollah Mohseni & Mohsen Safaei

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LETTER TO THE EDITOR

The multifunctional nano-immunoliposome design: hypothesis on a therapeutic approach for COVID-19

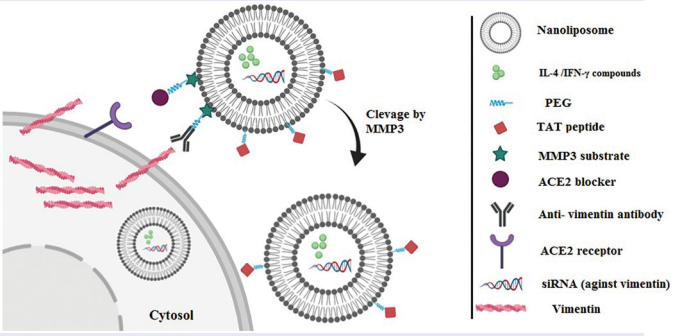
Zahra Abpeikar[a], Roohollah Mohseni[b] and Mohsen Safaei[c]

[a]Department of Tissue Engineering, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran; [b]Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran; [c]Department of Medical Biotechnology, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran

ABSTRACT

The article presents a hypothesis on a co-delivery strategy to suppress or reduce infection caused by the COVID-19 virus. Co-delivery was illustrated in many diseases, and results showed that it produced a high therapeutic efficacy against disorders. We proposed an approach to suppress or reduce infection caused by the coronavirus disease 2019 via designing an intelligent nano-liposome loaded with interferon γ, interleukin 4 and small interfering RNA against vimentin. At the surface of this nanostructure, there is a matrix metallopeptidase3 substrate to provide a platform for the enzymatic function of matrix metallopeptidase3 to destroy the extracellular matrix, angiotensin-converting enzyme 2 blocker, and antibody against vimentin for targeting, trans-activator of transcription peptide, and polyethylene glycol. Due to the increasing application of nano-liposomes commercially as a drug-delivery system, it is important to consider this effective approach for the coronavirus disease 2019 treatment.

GRAPHICAL ABSTRACT



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CONTACT Mohsen Safaei mohsensafayi@yahoo.com, st-safaei.m@skums.ac.ir Department of Medical Biotechnology, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran

Discussion

The recent outbreak of coronavirus disease 2019 (COVID-19), the novel disease arising from a new coronavirus species, named SARS-CoV-2, has caused many researchers around the world to find treatment for this condition. Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causing severe respiratory diseases, two other known viruses from the same genera, were identified in 2002 and 2012, respectively. COVID-19, as a novel virus, seems to have a similar pattern to SARS and MERS (Mosaddeghi et al. 2020). Innate immune system interactions with the viral play a key role in determining the outcome of infection. Early control of viral replication by type I interferons (IFN-I), complement proteins, and other innate immune mediators limit viral spread within the host during the early phases of the disease (Frieman, Heise, and Baric 2008). IFN-I (IFN-α and IFN-β) has a protective effect on SARS-CoV and MERS-CoV infection, but the IFN-I pathway in infected mice is inhibited (Li et al. 2020). Therefore, preventing IFN-I inhibition or loading it into the cell can effectively reduce the virus's infection.

Angiotensin-converting enzyme 2 (ACE2), the type I integral transmembrane protein, was considered the functional receptor for SARS-CoV both *in vitro* and *in vivo* (Yu et al. 2016). Researches in the expression of ACE2 protein, cellular distributions, and tissue tropism of SARS-CoV, provided novel insight into the mechanism of pathogenesis. On the other hand, vimentin has been reported to play roles in viral multiplication; it interacts directly with SARS-CoV spike protein during the spike-ACE2 binding process and serves as a putative co-receptor involved in the entry of SARS-CoV. Vimentin is the major component of type III intermediate filament protein. Its main purpose is to maintain the architecture of the cytoplasm. It can also be secreted under certain conditions and participates in cell adhesion, migration, and cellular signaling (Yu et al. 2016). Thereby, it seems that controlling or reducing these receptors' activity is effective in the treatment of viral infections.

Various delivery systems, such as viral and non-viral vectors, have been introduced, which has a promising role in gene/drug therapy's success. Non-viral delivery systems reduce immunogenicity and carcinogenesis, so they are desirable candidates for gene/drug therapy due to their well-defined physical and chemical composition. In this regard, cationic lipids and liposomes display promising advantages, including low toxicity, efficient interaction with lipid cell membranes, and enhanced endosomal escape. They have been known as suitable carriers for gene/drug delivery (Mura, Nicolas, and Couvreur 2013).

Presentation of the hypothesis

Many researchers have started to explore effective treatment strategies against COVID-19 diseases, Such as discovering effective drugs and vaccines. However, the important role of nanotechnology must also be considered. We proposed an approach to suppress or reduce infection caused by the COVID-19 virus via designing an intelligent nano-liposome loaded with interferon γ, interleukin 4, and small interfering RNA (siRNA) against vimentin. At the surface of this nanostructure, there is matrix metallopeptidase3 (MMP3) substrate [to provide a platform for the enzymatic function of MMP3 for the destruction of extracellular matrix (ECM)], ACE2 blocker and antibody against vimentin (for targeting the desired cells), trans-activator of transcription (TAT) peptide and polyethylene glycol. The MMP3 substrate, in addition to its important role in destroying the ECM, also provides a connection site for ACE2 blocker and antibody against vimentin, due to the effects of IFN-I (IFN-α and IFN-β) on SARS-CoV and MERS-CoV infections, and inhibition of IFN-I pathway in infected mice, therefore, IFN-I can also be loaded in nano-liposomes or a mixture of two types of nano-liposomes can be used, one with IFN-I and the other with IFN-γ and IL-4. In the following, the reasons for using each of these compounds are explained.

In this regard, a virus-infected cell model has been assumed, and in parallel, phospholipids have been covalently conjugated to TAT peptide, MMP3 substrate, anti-vimentin antibody and blocker for ACE2 receptor. The functional lipids have been mixed to generate intelligent liposomes containing siRNA and IL-4/IFN-γ compounds with high efficiency, which could be a promising strategy for the treatment of COVID-19. Several synthesis steps of selected moieties perform the fabrication of intelligent liposomes with TAT peptide and anti-vimentin antibody to PEG-conjugated lipids. The TAT peptide enhances the intracellular uptake and lysosomal escape of the nano-vesicles and increases the intracellular loading of selected siRNA (against vimentin) and IL-4/IFN-γ compounds. Previous studies have shown that cytoplasmic vimentin is translocated to the cell surface and interacted with the spike-ACE2 complex, revealing a putative entry pathway for SARS-CoV and other viruses (Du et al. 2014; Yu et al. 2016). Therefore, suppressing vimentin (Both intracellular and on the cell surface) through siRNA and anti-vimentin antibody probably disrupts the entry of the virus into the cell.

Different types of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are used for cardiovascular diseases. It is believed that ACEIs and ARBs increase the numbers of ACE2 receptors. Several studies have suggested that ACE2 receptors serve as binding sites for the anchoring spike proteins on the exterior

surfaces of COVID-19. Accordingly, it has been hypothesized that patients treated with ACEIs and ARBS, due to increased numbers of ACE2 receptors, may be at increased risk for COVID-19 infection (Diaz 2020; Li, Hu, and Zhang 2020; Zheng et al. 2020). On the other hand, a study by De Lang, et al. in 2006 showed that treatment of Vero E6 cells with interleukin-4 (IL-4) and Interferon-γ (IFN-γ) downregulate the expression of the SARS coronavirus receptor ACE2 (de Lang, Osterhaus, and Haagmans 2006). Since COVID-19 exhibits a very high sequence homology with SARS-CoV-1, these results can be used for COVID-19 (Kim et al. 2020). Because we have to use ACE2 receptors blocker at the surface of the nano-liposome for targeting, we can use IL-4 and IFN-γ within the nano-liposomes to neutralize the effect of the blocker on increasing receptor expression in the target cells.

The studies have shown that coronaviruses inhibit the IFN-I pathway in infected mice. On the other hand, nuclear transport of IFN regulatory factor 3 (IRF3) is suppressed by MERS-CoV (Frieman, Heise, and Baric 2008; Li et al. 2020; Yang et al. 2015), thereby loading of interferon into the nano-liposomes may have a protective effect on SARS-CoV2 infection, but here, first of all, our goal is to block the ACE2 receptor and then to prevent the increased expression of these receptors by the target cells, so we can use IL-4/IFN-γ compounds within the nano-liposomes to neutralize the effect of the blocker on increasing receptor expression in the target cells.

The MMP3 are zinc-dependent endo-peptidases and comprise a large family of enzymes with different abilities to degrade specific ECM components. Therefore, the use of the MMP3 substrate at the surface of the nano-liposome provides a platform for the enzymatic function of MMP3 to destroy the ECM. Incorporation of anti-vimentin antibody and ACE2 blocker on the surface of liposomes leads to specific targeting of infected cells through vimentin, and ACE2 receptors and the presence of MMP3-cleavable substrate provides enhanced conditions to expose TAT-linked liposome uptake. On the other hand, co-immunoprecipitation analysis suggested that MMP3 directly interacted with NFkB in the nucleus during DENV infection in Vero E6 and other cells and showed antiviral activity against DENV infection (Zuo et al. 2014). Therefore, the study of MMP3 anti-viral activity in COVID-19 infectious cells requires investigation. PEG is also used in the design of nano-liposomes. Medium-sized PEG linkers stabilize liposomes and avoid them from aggregation and without visible steric hindrance, provide sufficient spacer length for the functional TAT, MMP3, and antibody moieties to interact with their respective targets (Xia, Tian, and Chen 2016). Our goal is targeted liposomes production that may become a potential tool in gene/drug delivery

to improve disease control or reduce the activity of factors involved in the signaling of COVID-19 viral infection.

Testing the hypothesis

For this study, vero E6 cell line, siRNA, IL-4/IFN-γ compounds, anti-vimentin antibody, ACE2 receptor blocker, and also, lipid compounds including Cholesterol, L-α-phosphatidylcholine, egg, chicken (PC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2- dioleoyl-3 trimethylammonium-propane methyl sulfate salt (DOTAP), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethyleneglycol)-2000] (DSPE-PEG(2000)-maleimide) are supplied from a variety of sources. Optimized TAT and MMP3 peptide sequences are ordered from reputable companies. The next step, synthesis, purification, and verification of lipid/peptide compounds to bind to the nano-liposomes, will be performed. The hypothesis can be investigated by encapsulating siRNA and IL-4/IFN-γ compounds into liposomes using the thin-film hydration method; then the following steps are performed: (1) Micelle generation and antibody/blocker conjugation, (2) Formulation of initial non-targeted liposome containing siRNA or IL-4/IFN-γ compounds, (3) Formulation of targeted liposomes (4) Cell toxicity, cellular uptake, and competition analysis. It is important to note that the insertion of the antibody/blocker conjugated micelles into initial liposomes is applicable by the post-insertion method. Cytotoxicity effects of nanoparticles will be evaluated after 5 days, using the MTT assay; also, cellular uptake analysis and gene/protein quantification (including ACE2 and vimentin) are accomplished using flow cytometry, real-time PCR, and western blot techniques, respectively.

Implications of the hypothesis

Cationic lipids and liposomes demonstrate promising advantages, such as low toxicity, efficient interaction with lipid cell membranes, and enhanced endosomal escape. They have been known as suitable carriers for gene/drug delivery (Zhi et al. 2018). Nano-liposomes are considered both as an investigational system and commercially as a drug-delivery system. Many studies have been conducted on liposomes to decrease drug toxicity and reduce non-specific cells' targeting (Akbarzadeh et al. 2013). The conjugation of cell/tissue-specific ligands on the surface of liposomes efficiently has influenced the potency of gene/drug delivery. Targeted liposomes improve the tendency to load in infected areas, minimize unspecific delivery to other tissues, and facilitate cell-specific treatment. Co-delivery was illustrated in several diseases, including cancer by

many researchers, and results showed that it produced a higher therapeutic efficacy against infections. (Perche and Torchilin 2013). This hypothesis focuses on the generation of nano-liposome with properties co-delivery of siRNA and IL-4/IFN-γ compounds against the virus-infected cells. On the other hand, selected key factors (such as an anti-vimentin antibody, ACE2 blocker, and MMP3 substrate) from various articles to targeted nano-liposomes fabrication confirms that the targeting of the infected cells or areas will be carried out effectively.

In this investigation, a virus-infected cell model is considered, and in parallel, phospholipids have been covalently conjugated to TAT, MMP3, anti-vimentin antibody and blocker for ACE2 receptor. The functional lipids have been mixed to generate intelligent liposomes harboring siRNA and IL-4/IFN-γ compounds with high efficiency, which could be a promising strategy for the treatment of COVID-19.

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Author contributions

All authors collected and analyzed relevant literature, then drafted the manuscript, critically revised the manuscript for content, and finally read and approved the final manuscript.

Disclosure statement

The authors report that there are no conflicts of interest.

ORCID

Roohollah Mohseni http://orcid.org/0000-0001-9759-0876
Mohsen Safaei http://orcid.org/0000-0003-1504-5490

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